SOME EFFECTS OF TEMPERATURE ON THE POLLEN OF *ORNITHOGALUM CAUDATUM*

by

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B. S., Kansas State University, 1965

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Botany and Plant Pathology

Kansas State University
Manhattan, Kansas

1967

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INTRODUCTION

In pollen germination studies it has been observed that *in vitro* germination percentage of pollen obtained from plants grown in the greenhouse in summer has been much less than from the same species grown during the cooler seasons of the year. This phenomenon did not appear to be limited to one species, but appeared to be rather common. Since high temperature may have been the causal agent, this study was undertaken to determine if plants grown in an environment of high temperature did produce a lower viable pollen percentage, and if so, what could be done to alleviate this injury.

The interaction between temperature and pollen germination has been studied by several investigators for many years. Using germination percentage *in vitro* as a test for viability, it has been generally determined that the optimum temperature for the germination of control pollen is about 28°C. At higher and lower temperatures, viability decreases. Since daytime temperatures often exceed this value during the summer in the field, it is surprising that plants are as efficient as they are in fertilization. Indeed, it has been known for several years that corn ears often do not fill during hot summer months due to the effects of temperatures above optimum on the pollen. However, critical studies of pollen viability as a physiological phenomenon have not been extensively reported.

SURVEY OF LITERATURE

Morphological Stages and Physiological Changes During Pollen Development

Morphological studies of pollen development in Ornithogalum caudatum appear not to have been reported. However, such studies with other species have been conducted. Erickson (1947) working with Lilium longiflorum observed that pollen mother cells underwent meiosis approximately twenty-five days before anthesis. Mitosis of the microspore nucleus occurred about twelve days prior to anthesis. These observations were made from February 11 to April 8 at Rochester, New York. The plants were grown under greenhouse conditions where the temperature fluctuated daily from about 16°C to about 27°C.

Beatty and Beatty (1953) stated that microgametogenesis from meiosis through pollen maturation required about fourteen days in Tradescantia paludosa at 30° ± 0.5 Their observations were made on potted plants during the summer months at Emory, Georgia. A very detailed timing sequence for meiosis and microspore development was given.

Kiyosawa (1962) found that meiosis in pollen mother cells occurred thirteen days before heading in pot grown rice plants. The daytime temperature was 19°C and the night temperature was 15°C in his studies.

Heslop-Harrison (1964) working with Cannabis sativa and Silene pendula made the following observations on pollen wall development. The archesporium and pre-meiotic cells produced

only a primary wall of cellulose. This cellulose wall continued to be formed through the tetrad stage. A special wall of callose was initiated in early meiosis and became more prevalent as meiosis continued. The synthesis of this layer predominated wall development from meiosis through the tetrad stage. During the tetrad stage a primexine wall was produced which he thought was primarily cellulose. This wall established the pattern to be imprinted in the exine. The tetrad then broke up and an exine of sporopollenin was deposited on the microspore. In the latter stages of exine formation, an inner wall of cellulose, the intine, formed. Then anthesis occurred.

Taylor (1959) and Takats (1962) concluded that the tapetum was the source of the microspore exine. Rowley (1964) presented the idea that exine development may be connected with substrate transfer from the tapetum to the microspores. His support for this comes mainly from electron photomicrographs that showed protoplasmic connections of the microspore protoplast with the exine through the intine.

Physiological changes have also been reported during pollen development. Kiyosawa (1962) found that in rice a night temperature of 15°C produced a significant loss of starch in the mature pollen. The developing pollen was sensitive to this lower temperature at two different stages. The first was during meiosis and the second was during exine development. Also, Britikov et al. (1964b) observed proline was translocated from vegetative tissues and started to accumululate in the developing microspores in the tetrad stage of Papaver somniferum and Lilium superbum and

continually increased as the pollen developed.

Stern (1947) studied the permeability of developing pollen mother cells of Trillium erectum. These cells were most permeable just prior to meiosis and just after mitosis. Permeability was determined by the rate of uptake of several stains and was assumed to be passive permeability. Stern stated that the magnitude of change was probably the greatest recorded for plant or animal cells.

Changes in respiration rate with stage of pollen development have been reported. Erickson (1947) reported on the sequence of division and respiration rates of pollen mother cells of Lilium longiflorum. The respiration rate dropped from 731 to 314 mm^3 O_2/hr./gm. during microsporogenesis. Two pronounced decreases in rate occurred at the times of meiosis and of microspore mitosis. Stern (1947) determined oxygen consumption of single Trillium anthers. There was a steady rise in oxygen consumption prior to mitosis, a sharp drop just preceeding active division, and a low rate during division, but the rate increased toward the termination of the cycle.

Nasatir et al. (1960) found that the soluble proteins from Lilium anthers contained four electrophoretic components. One increased in amount before mitosis and disappeared at mitosis: two had peak values during mitosis and decreased thereafter: while the fourth component remained relatively constant in microspore development.

Linskens and Schrauwen (1963) found the sulfhydryl content of Lilium pollen mother cells showed synchrone maxima in pachytene

and after tetrad formation. Transfer of SH groups from the tapetum to microspores during metaphase II was presumed to occur. Changes in SH content during meiosis and pollen formation were not observed.

Bolkhavskikh (1965) reported that changes during pollen development in Lilium were connected with changes in metabolism and localization of DNA. Also RNA content was reduced in the chromosomes during late prophase of the microspore nucleus. During this time, RNA migrated into the cytoplasm. At prometaphase part of the nucleolar RNA moved to the chromosomes and remained associated with the chromatids until late telophase.

Effects of Temperature on Germination in vitro

Johri and Vasil (1961) reported that Roberts and Struckmeyer (1948) and Visser (1955) represented the correlation between temperature and pollen germination with an optimum curve. Maximum germination and tube length were obtained between 20°C and 30°C (Winkler, 1926; Berg, 1930; King and Johnston, 1958). However, Hirose (1957) reported that the optimum temperature for pollen germination in tobasco variety of red peppers was 35°-40°C. This was unusual since germination was usually inhibited at these temperatures (Johri and Vasil, 1961). At lower temperatures, Ostlind (1945) found that in apple there was a reduced rate of germination and tube growth. At 20°C after sixty-nine hours, germination was 90-100 per cent.

With an increase in temperature above the optimum, pollen

tubes increased in diameter and further temperature increases caused the growing tips to burst (Sen and Varama, 1956; Vasil and Bose, 1959).

Causes of High Temperature Injury

The effects of temperature on intact plants, as well as on individual plant cells, are quite complex, since all physical and chemical phenomena are affected. Meyer et al. (1960) stated that in general an increase in temperature decreases the solubility of a gas, increases the speed of diffusion, increases osmotic pressure of a solution, increases the rate of transpiration, directly affects the rate of enzyme controlled reactions, alters the stability of the enzymes, increases respiration rate, decreases the net daily gain in photosynthesis, inhibits reproductive development, may stop plant growth, or even kill the plant and probably has a variety of other effects. Temperature induced deficiencies of essential metabolites are well known from many references given in a review by Langridge (1963).

The ability of a plant to resist heat injury is often an indication of the mechanics of heat injury. Khlebnikova (1932, 1934, and 1937) stated that there were two kinds of heat resistance in the plants with which he worked. His studies demonstrated one modification was an extensive root system and an intensive transpiration rate which was typical of watermelon. The other was that the protoplasm simply survived overheating which was typical of pumpkin. However, the ability of protoplasm to

survive overheating was not a simple phenomenon.

Kruzhilin et al. (1951) proved that nonprotein nitrogen and ammonia accumulated and protein nitrogen decreased during the hot hours of the day in heat-intolerant plants. Petinov and Molotkovsky (1957) said heat resistant plants subjected to high temperatures had reduced respiratory coefficients and accumulated organic acids which reacted with ammonia to form salts and amides which was a protective reaction against ammonia toxicity. Petinov and Molotkovsky (1960) also reported that certain respiratory inhibitors (KCN, monofluoroacetate, arsenite, and 2,4-dinitrophenol) caused plants to be susceptible to heat damage. Amino acid and amide synthesis declined and oxidative phosphorylation was reduced. Petinov and Molotkovsky (1962) later described methods of overcoming heat damage by infiltrating plants with organic acids and the addition of zinc which stimulated dehydrogenase activity and increased oxidative phosphorylation. Henckel (1964) reported that it had been shown that high temperatures caused the hydrolysis of complex proteins with an accumulation of amino acids.

Nucleic acids have also been implicated in heat resistance. Tabor (1962) observed that the addition of amines to calf thymus DNA mardedly increased the temperature requirements for the denaturation of the DNA. Similar studies with plants are lacking.

From the review of Langridge (1963) one is led to conclude that there are many aspects of protoplasmic heat resistance and that heat resistance corresponded to thermostability of the proteins and varied from species to species.

There are a number of physiological responses to increased

temperature which are the same for drought. Julander (1945) indicated that heat resistance of several species of range grasses corresponded closely with the aridity of their natural habitat and were apparently a measure of drought resistance. Henckel (1946) established that plants responded to dehydration caused by drought by increasing the hydration and hydrophily of their protoplasmic colloids. Later, Henckel (1956) and Satarova and Ulybina (1960) indicated that heat resistance was closely associated with certain colloidal-chemical properties and with extent of hydration. Henckel and Lyubimova (1947) previously had shown the amount of bound water and the degree of protoplasmic viscosity to be also associated with heat resistance. Dessication from drought, as well as from high temperatures also caused a destruction of protein molecules, according to Lepeschkin (1938) and high temperature stimulated the conversion of starch to sugar (Meyer et al. 1960) as did drought (Julander, 1945).

Drought and high temperatures have been associated with smaller pollen grains. Schoch-Bodmer (1940) and Anikiev (1960) reported that high air temperature during the night and a deficiency in water, reduced the size of pollen at maturity. Wagenitz (1955) observed that drought decreased pollen diameter in wheat, rye, and oats. Kurtz and Liverman (1958) demonstrated that both high and low night temperatures and high day temperatures decreased diameter of pollen from tomato and cocklebur.

Changes in respiration during drought have been reported. According to Lvov and Fichtengolz (1936), mesophytic plants had a higher respiration rate during drought than when soil moisture

was optimum. They thought it was possible that the plants would maintain the water content of the protoplasmic colloids with the water released by the intensified respiration. Bennet-Clark and Bexon (1943) found that in plasmolyzed beet root disks, respiration increased initially but later fell below the normal respiration rate. Petinov and Molotkovsky (1956, 1957) and Altergot (1960) associated drought and heat resistant plants with an increased capacity for protein synthesis and not with snythesis of amides. They concluded that respiration must be productive in oxidative phosphorylation when influenced by high temperature. Henckel (1964) stated that it was low and not high metabolic activity which stimulated formation of increased amounts of organic acids.

Drought or high soil moisture stress has been shown to alter plant development. Konarev (1959) found that phosphorus nutrition, light, and water all stimulated the development of xeromorphism. All these factors affected the same aspect of plant metabolism, namely, nucleic acid metabolism and altered tissue differentiation. Water deficiency always accelerated the differentiation of tissue and caused a decrease in cell size. Shaw and Loomis (1965) found increased moisture stress to be associated with decreased adenylic and increased uridylic acid contents. During stress, soluble RNA increased and ribosomal RNA decreased. They concluded that changes in growth caused by moisture stress may be related to RNA and protein metabolism.

Temperature injury had been shown to be associated with both cytological and metabolic alterations in pollen. Nakahara

and Komoto (1957) applied high temperature to the excised anthers of Tradescantia paludosa, and observed various chromosomal aberrations such as swelling, clumping, stickiness, missdivision, and other abnormalities to occur during initial meiosis. Kiyosawa (1962) observed a significant loss of starch in mature rice pollen due to a night temperature of 15°C and a day temperature of 19°C. There were two stages in pollen development that were particularly sensitive to low temperature injury. One occurred about thirteen days and the second about ten days before heading. These stages seemed to correspond to the stages of meiosis and of exine formation. A decrease in photosynthesis during the cold treatment was not the cause of the injury.

Loss of pollen viability due to high temperatures has been reported. Abaeva (1941) found that in cotton plants at 41-42°C non-viable pollen was produced, but at 35-37°C pollen was viable. Viability was determined by germination on the stigmas of excised flowers. Jones (1947) germinated corn seeds at 30°C. When the shoots were one-fourth to one-half inch long, three lots were exposed to temperature treatments of 40, 50, and 60°C for one hour. All treated seedlings were shorter in height, less vigorous in growth, later in flowering, and produced nonviable pollen.

MATERIALS AND METHODS

Plants and Pollen

Ornithogalum caudatum is a bulbous member of the Liliaceae.

It is native to South Africa, Bailey (1916), and is sometimes grown as a house plant. Goss (1962) working with this species concluded it was ideally suited for pollen germination studies. The pollen germinates rapidly and percentage germination is high. The inflorescence is a raceme and stays in flower for two or three months and may contain twenty flowers in anthesis at one time. The pollen used here was fresh pollen obtained from plants cultured in growth chambers or greenhouses.

Nutrient Medium and Germination Techniques

The nutrient medium, consisting of a sucrose concentration of 10%, 1.26 mg/ml of boric acid, and 5 ml of a stock macronutrient solution per 10 ml, gave optimum germination percentage for Ornithogalum caudatum pollen (Goss, 1962). The macronutrient solution was prepared from 2.68 gm of $Ca(NO_3)_2 \cdot 4H_2O$, 8.0 gm of KNO_3, 1.0 gm of KH_2PO_4 and 6.08 gm of $MgSO_4 \cdot 7H_2O$ dissolved in water and diluted to one liter.

The pollen was germinated by a hanging drop technique using petri dishes. A grease pencil was used to make small circles on the inside of the cover on the dish. A drop of nutrient medium was placed in each circle, pollen grains were added to the medium, then the cover was inverted and placed over the bottom of the petri dish which contained about 2 ml of water. This porvided a humid environment for the pollen and reduced evaporation from the culture media. The petri dishes were then placed in an incubator at 30°C for 30 minutes, and photomicrographs made or permanent

slides prepared.

Conditions of Exposure

Unless otherwise indicated, all plants used were grown in growth chambers. The term high temperature, as used here, means a temperature of 31°C ± 1°C during the daytime, and 29°C ± 1°C at night. The day length was fourteen hours with a ten hour night period. The light intensity was approximately four thousand foot-candles at the level of the flowers on the raceme which were eight to ten inches from the light source. There was no humidity control. The relative humidity varied with temperature and the number of plants in the chamber, but remained approximately 65% during the day and 75% during the night. The control temperature was 25°C ± 1°C with a relative humidity of 30% during the day, and 45% at night. The term greenhouse temperature, as used here, was near control temperature, otherwise the temperature and humidity are stated. The plants were watered as needed.

Botanical Microtechnique

The meiotic sequence and pollen development were studied from permanent slides. Flower buds were sectioned longitudinally and transversely, twelve microns thick, and mounted in permount in serial section form. The inflorescence was killed and fixed in Carnoy's fluid and stained with Harris' hematoxylin, safranin, and fast green, using the techniques given by Johansen (1940). This stained the nuclear material red, lignified tissues red, and

other structures green. A number of other slides were made using a modification of a technique given by Beeks (1955). Whole anthers, squashed anthers, pollen, and germinated pollen were stained with acetocarmin and mounted in Hoyer's medium.

Respiration Techniques

Respiration rates were determined for pollen collected from several treatments. The technique employed was adapted from Grunbaum et al. (1955). The apparatus used was a differential ultramicrorespirometer. The only modification was setting up the apparatus at 23°C and then placing the flasks in a 30°C water bath for the oxygen determination. The technique described by Grunbaum et al. (1955) used a walk-in incubator and room temperature was the same as the water bath temperature. The respiration rates of 0.2 mg to 0.8 mg of pollen were measured and reported in ul O_2/mg fresh pollen/hour.

Gravimetric Determination of Moisture Per Cent

Moisture content was determined for different plant material. These tissues were placed either in a paper bag or in aluminum foil weighing cups and weighed. They were then dried at 100°C and reweighed. The cups or bags were then weighed and moisture content reported to the nearest tenth of 1%. Weights were recorded to the nearest 0.1 mg.

RESULTS

Preliminary Observations

Development of the pollen of O. caudatum was studied using the slides prepared as previously described. At the control temperature, microspore mother cells were formed from the sporogenous tissue, and meiosis occurred approximately twenty-two days before anthesis (Fig. 1). Microspore mitosis occurred about eight days before anthesis when the microspores were readily plasmolyzed and apparently contained a very high content of water (Fig. 1). After this time, they were less easily plasmolyzed and contained more solid material. This observation was made on the material which was sectioned, dehydrated, stained with safranin, redehydrated, stained with fast green, and mounted in permount in the dehydrated condition. Thus, it was concluded that increase in dry matter of the microspore occurred mostly from eight days on to anthesis. The exine began its greatest development ten days prior to anthesis, about two days before mitosis. The exine developed completely following mitosis, following which anthesis occurred (Fig. 1).

This time sequence of days prior to anthesis was developed by comparing the rate of flowering of several plants with the number and location of sectioned buds and anther squashes of other flowers. This sequence was then considered valid for the specific conditions in the control growth chamber and would vary with other conditions, especially temperature, but was an estimate of the time these events occurred (See Fig. 2 for the comparison of the rates of anthesis at the control temperature and at high

Fig. 1. Stages in pollen development in *O. caudatum*

A. Mature microspore mother cells twenty-three days before anthesis (magnification 970x).

B. Microspore mother cells during metaphase I, twenty-two days before anthesis (magnification 970x).

C. Microspore mother cells in anaphase I and telophase I, twenty-two days before anthesis (magnification 970x).

D. Microspores at the time of microspore mitosis, eight days before anthesis (magnification 100x).

E. Mature pollen at anthesis (magnification 430x).

F. Microspore mother cell formed at high temperature, fifteen days before anthesis (magnification 970x).

G. Pollen at anthesis produced under the influence of high temperature (magnification 430x).

H. Sporopollenin in a mature anther produced at high temperature (magnification 430x).

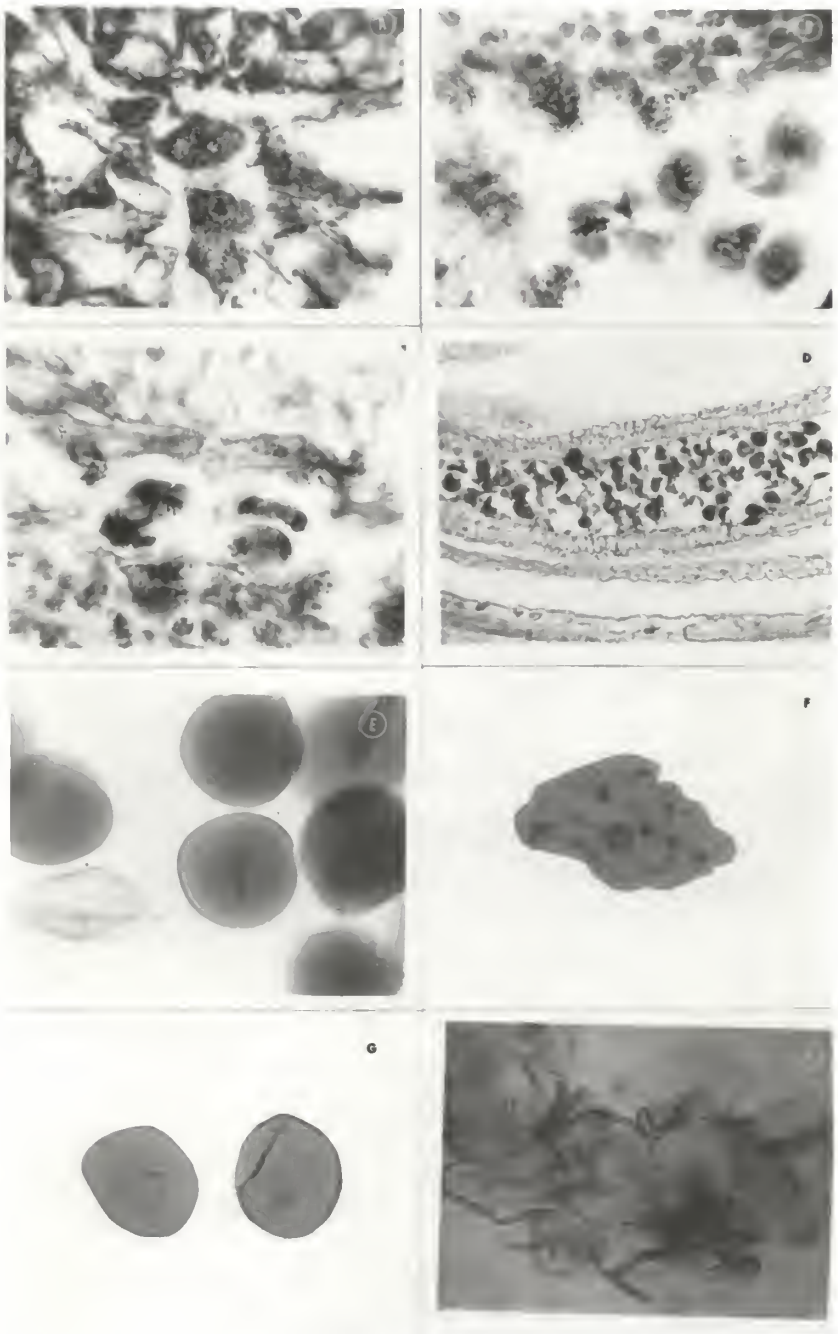


Fig. 1. Stages of pollen development in O. caudatum

temperature). This increase in temperature approximately reduced the interval from meiosis to anthesis by half.

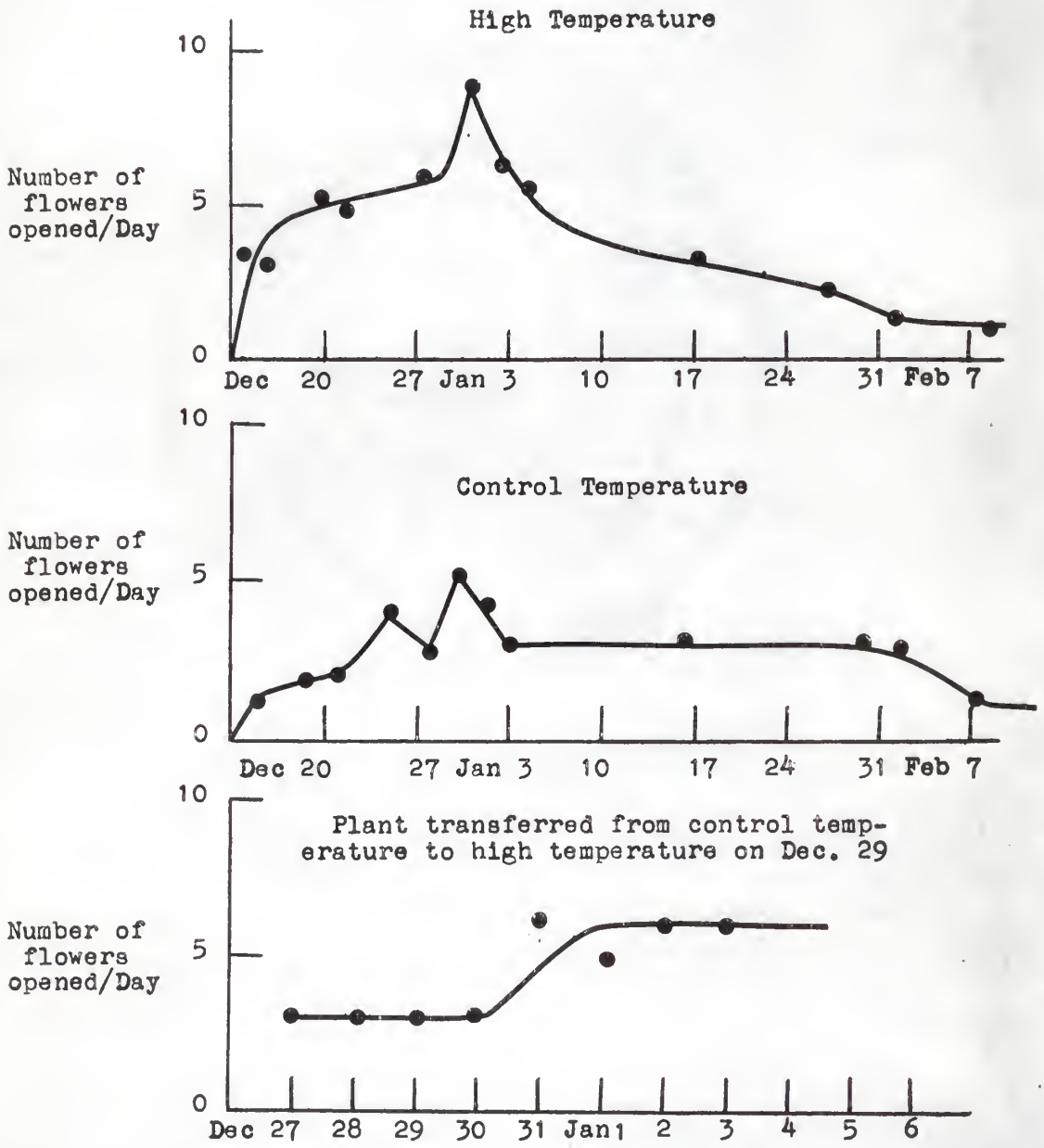
Respiration Data

Preliminary studies were made to establish the validity of the microrespirometer technique. It was necessary to prove that when the droplet in the manometer moved, the movement was due to a change in O_2 tension and not a change in other variables. It had been observed that if the respiratory flask contained nutrient medium and the compensation flask contained distilled water, the bubble moved slightly. Apparently the vapor pressure between the two was different. Therefore, in this study, both flasks contained nutrient medium.

If more nutrient medium was added to one flask than the other, and the apparatus was placed in the water bath, the droplet also moved. This possibly indicated a differential heating effect and consequently a differential in vapor pressure. Also there might be a difference in the quantity of atmospheric gases dissolved in the liquids. This droplet movement could be observed for as small a volume difference as one-tenth of one ul of nutrient medium. Therefore, it was considered necessary to use the same quantity of medium in each flask.

When pollen was dried at 100°C and placed in a respiratory flask, it was observed that the droplet moved quite readily. The pollen was hydroscopic and floated on the germination medium. After about fifteen minutes, part of the pollen sank and was rather

Fig. 2. Rates of anthesis of plants at high temperature, control temperature, and change in rate when transferred from control to high temperature.

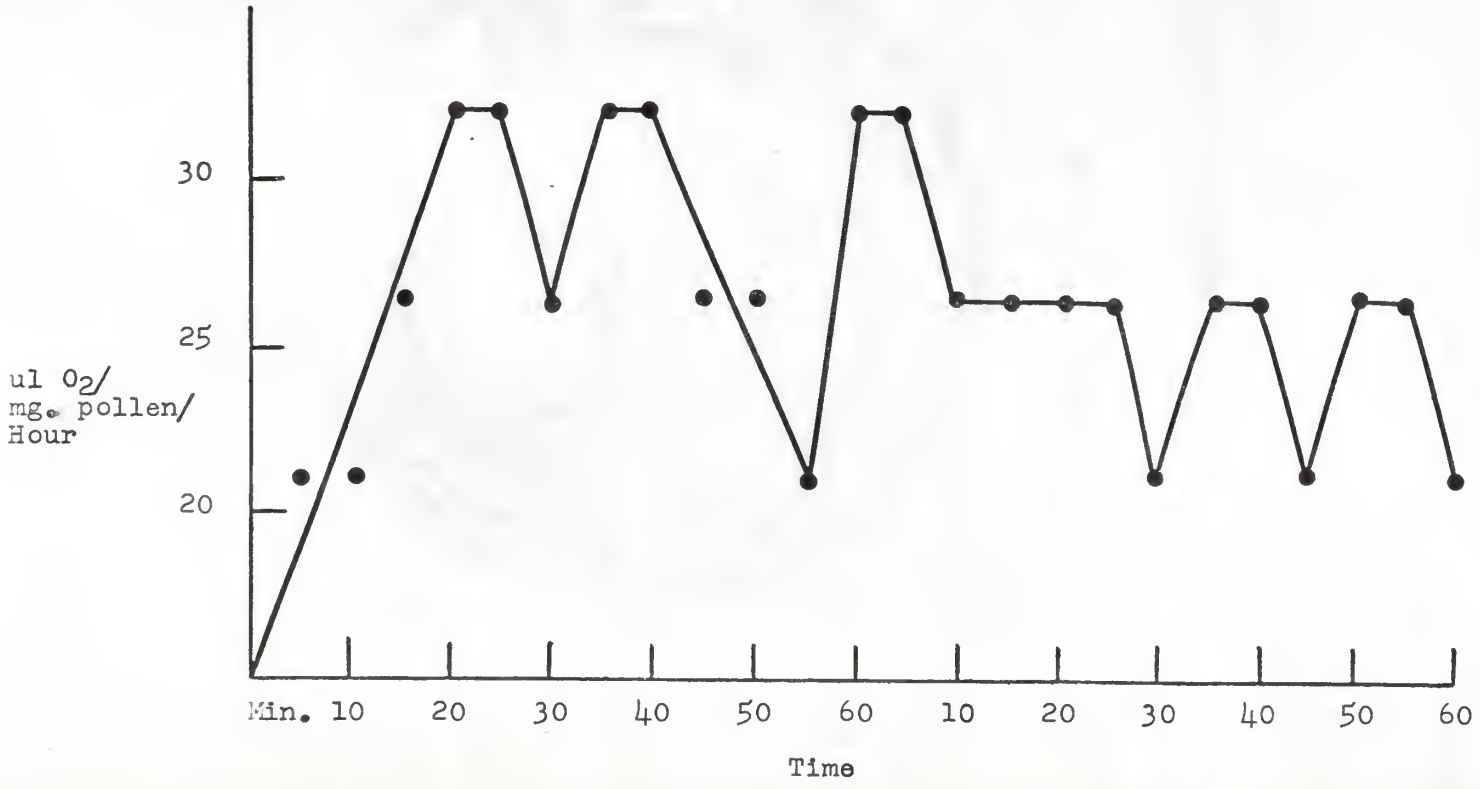


evenly dispersed throughout the culture medium. It is believed that the hydroscopic pollen may initially alter the vapor pressure and thus induce an error.

When using pollen dried at 100°C, a fifteen minute compensation period was allowed for the pollen to equilibrate with the vapor pressure in the respiratory flask before attachment to the monometer. This eliminated droplet movement. It was assumed then that any movement of the droplet under comparable conditions with viable pollen was due to changes in O_2 tension.

In order that the first fifteen minutes of respiration data were not lost while the pollen was equilibrating, a study was made maintaining the flasks at 10°C in the refrigerator. It was found that the rate of respiration of pollen at the end of thrity minutes was the same whether or not it had been in the refrigerator for a ten-minute interval. After removal of the flasks from the refrigerator, they were warmed by contact with one's fingers, attached to the mamometer, and placed in the water bath. The initial respiration rate was low but rose to its maximum rate in approximately fifteen minutes. Using heat-killed pollen under these conditions the droplet did not move. Thus the technique was considered valid. All respiration data were taken using this technique and presumably represent actual respiration. After attaining the maximum respiratory rate during the first fifteen minutes, the rate leveled off and remained approximately the same for the next two hours (Fig. 3). Hence, since respiration rates apparently did not change appreciably after the first half hour, this interval was adopted as the standard for experimentation.

Fig. 3. The respiration rate of a 0.5 mg sample of pollen collected July 16, 1965, from a plant grown under greenhouse conditions.



Using the most recently opened flowers on the raceme of one greenhouse-grown plant, a series of respiration experiments were made from July 14 through July 21, 1965. The pollen was collected at approximately 1:30 p.m. on each day. These respiration rates were compared with temperatures and humidity recorded in the greenhouse for those days by a hygrothermograph (Table I). The results indicated that a lower respiration rate on one day was correlated with a high temperature in the greenhouse approximately three days previously.

Table I. Effect of greenhouse temperature on respiration of *O. caudatum* pollen.

Date	Greenhouse Temperature*	Greenhouse Rel. Humid.**	Respiration Rate, ul O_2/mg pollen/hr.
July 10	82°C	66%	
11	81°C	74%	
12	90°C	60%	
13	88°C	64%	
14	78°C	54%	88
15	84°C	49%	30
16	90°C	52%	53
17	90°C	54%	66
18	86°C	62%	not taken
19	84°C	90%	38
20	90°C	85%	66
21	86°C	62%	53

*Highest temperature on that day
**Humidity at the highest temperature

The plant studied was placed in a temperature controlled environmental growth chamber on July 22. A treatment of ten hours night at 15°C and fourteen hours day at 25°C was used. Respiration rates from July 21 through July 29 are given in Table II.

Table II. Respiration rates of *O. caudatum* pollen.

Date	Respiration Rates, ul O_2/mg pollen/hr.
July 21, 1965	19
22, 1965	*not taken
23, 1965	16
24, 1965	21
25, 1965	not taken
26, 1965	31
27, 1965	30
28, 1965	33
29, 1965	31
Nov. 1, 1965	**28
2, 1965	44
3, 1965	30
4, 1965	35
5, 1965	44
6, 1965	42
Dec. 27, 1965	33
28, 1965	36
29, 1965	**39
30, 1965	29
31, 1965	39
Jan. 1, 1966	31
2, 1966	37
3, 1966	28
June 30, 1966	**10
July 1, 1966	18
2, 1966	21
3, 1966	27
4, 1966	21
5, 1966	37
6, 1966	23
June 30, 1966	***not taken
July 1, 1966	8
2, 1966	9
3, 1966	16
4, 1966	24
5, 1966	37
6, 1966	23

*Plant was transferred from high greenhouse temperature to the growth chambers at control temperature.
**Plant was transferred from control temperature to high temperature.
***Plant maintained continuously at control temperature.

It was observed that the pollen respiration rates increased when exposed to the cooler temperature. Results are not available from the third day the plant was exposed to the lower temperature, but on the fourth day the respiration rate was as high as the average respiration rate for the pollen produced at that temperature (Fig. 4).

Another respiration study was carried out from November 1 to November 6, 1965. The object of this study was to determine how the rate of respiration was affected when the plant was exposed to a temperature increase. Two plants were brought to flower at the programmed control temperature conditions and transferred to a growth chamber programmed for the high temperature conditions. The plant was transferred to the high temperature chamber on November 1, 1965. The respiration rates were erratic. There was a sharp increase the first day then a decrease the second, and third days, followed by an increase (Fig. 5). This further indicated something happening three days prior to anthesis. This was interesting because when keeping a plant at the high temperature and transferring it to the control temperature, the respiration rate of the pollen increased (Table II), which was the same observation for the plant kept at the control temperature then transferred to the high temperature. After several days exposure to high temperature, the respiration rate of heat resistant plants also decreased, according to Petinov and Molotovsky (1957).

Two more respiration studies were made and compared simultaneously with per cent germination. When a plant kept at the control temperature was transferred to the high temperature

Fig. 4. The change in respiration rate of mature pollen from plant grown at control temperature and transferred to high temperature on July 22, 1965.

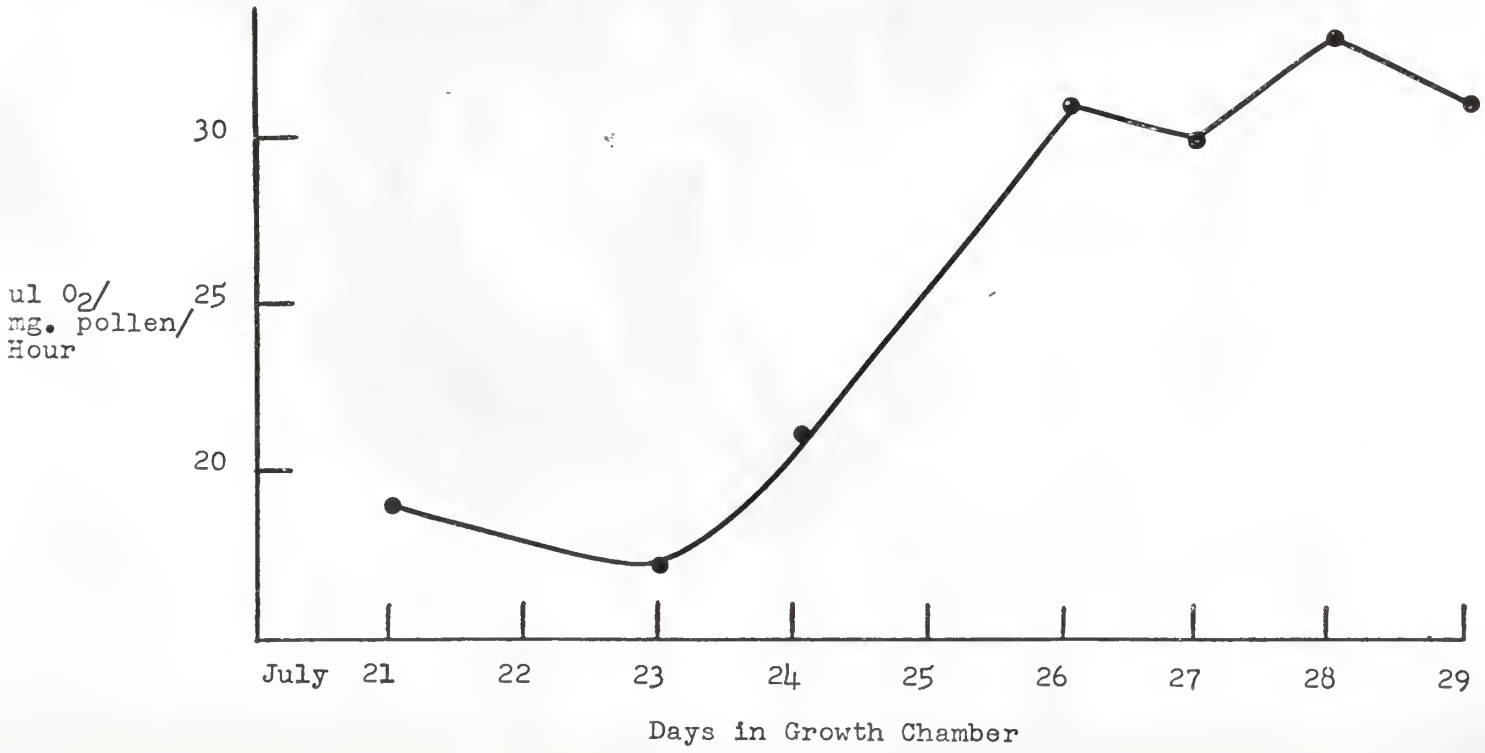
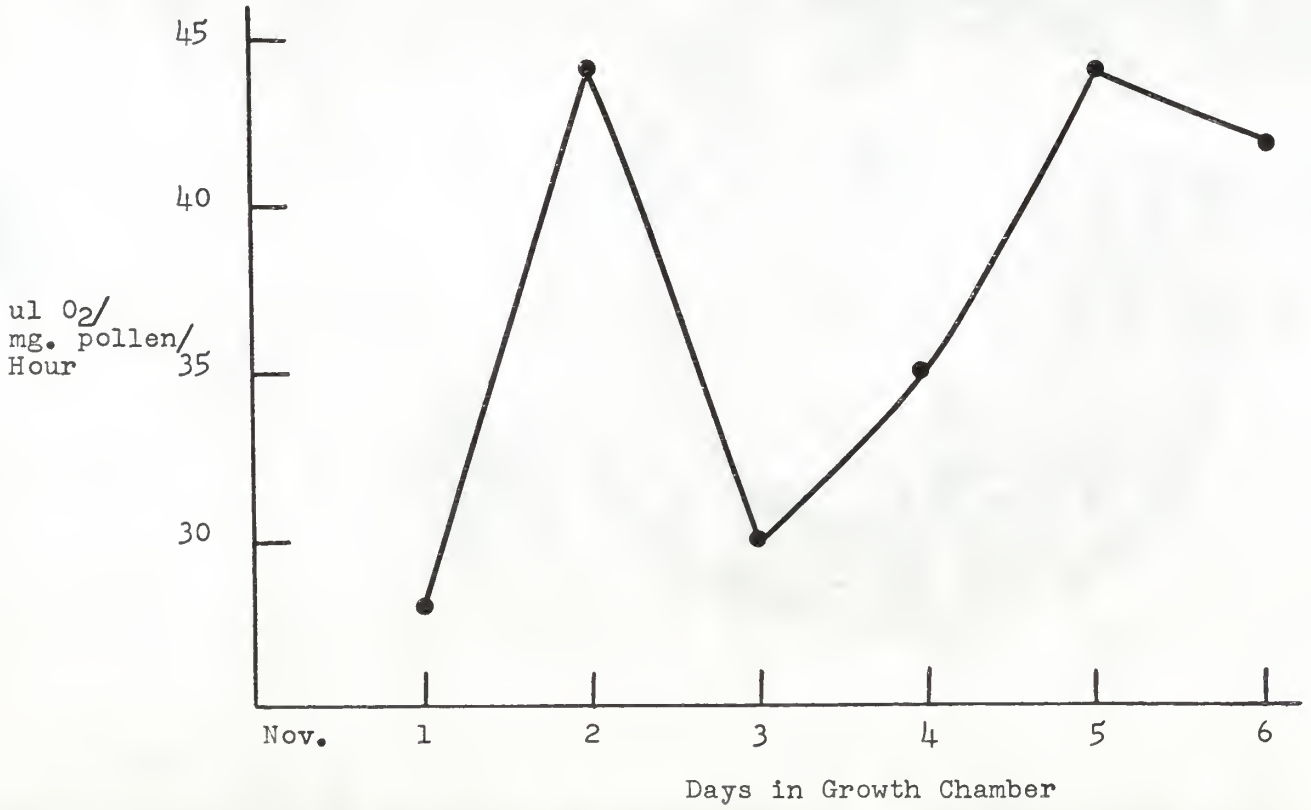


Fig. 5. The change in respiration rate of mature pollen from plant grown at control temperature and transferred to high temperature on November 1, 1965.



(Fig. 6) there was an overall increase in respiration rate of the pollen and a corresponding decrease in per cent germination.

Identification of Amino Acids and their Effect on Germination

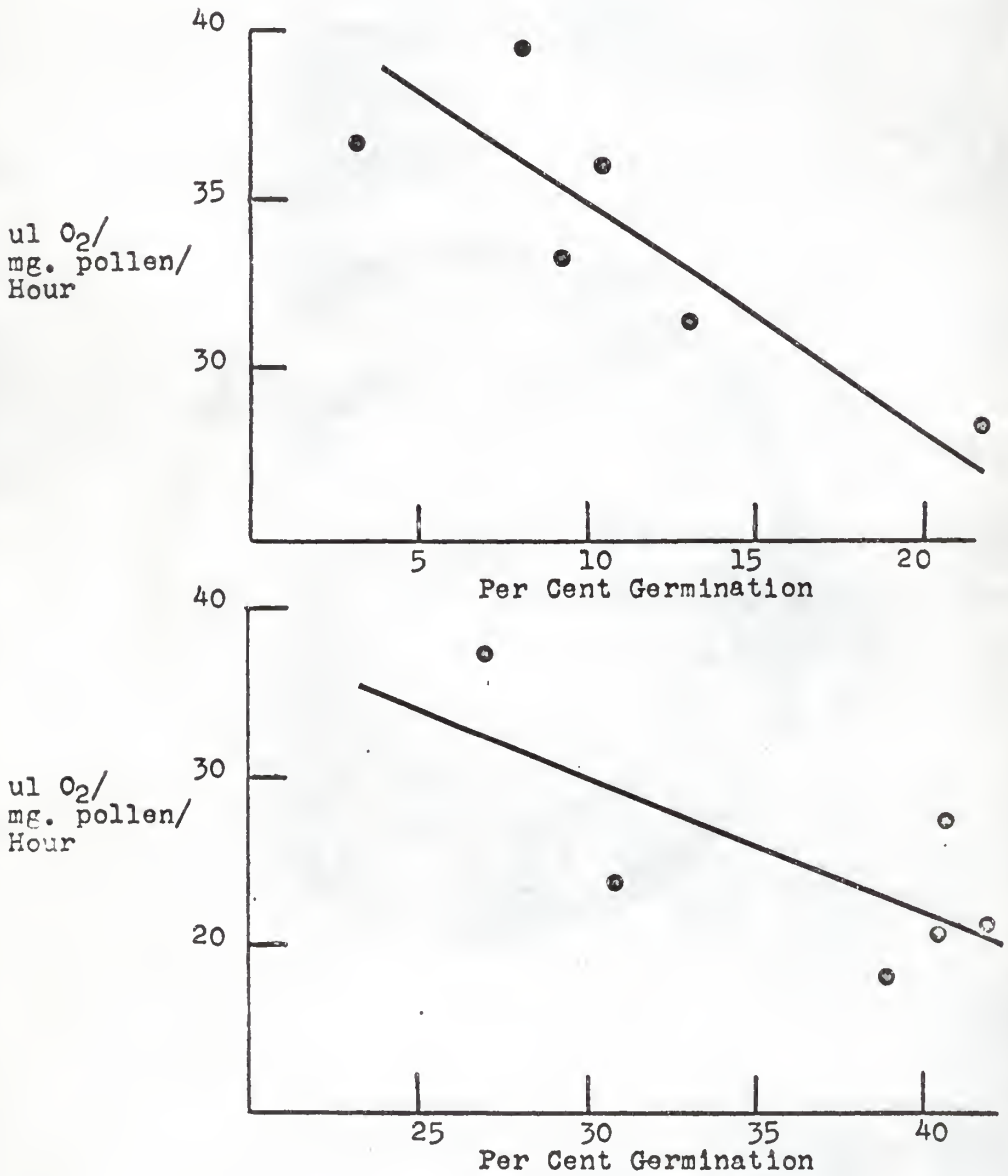
Free amino acids were determined in pollen produced at the higher temperature. A plant which was flowering was placed in a temperature controlled environmental growth chamber for a ten hour 23°C night and a fourteen hour 32°C day. Three days later all open flowers were discarded. Forty-eight hours later all open flowers (ca. 20) were removed. The pollen, styles, and stamens (washed free of pollen) were collected.

Free amino acids were extracted from these structures using 80% methanol for twenty-four hours at 10°C. Unidimensional paper chromatographs on Whatman No. 1 paper were run on this material using butanol, acetic acid, and water, in a 5:1:4 concentration ratio as the solvent. Proline and hydroxyproline were not observed in the pollen, but were identified in the other structures, particularly in the styles. Identification was made by comparison with known amino acid standards. Leucine and isoleucine were found in greater proportions in the stamen extract and were much less in the pollen extract.

Germination Data

The effects of the high temperature on germination percentage

Fig. 6. Comparison of Germination Per Cent with Respiration Rate.



have been shown (Fig. 6). The data from germination percentages were collected by two different methods. One involved a hanging drop culture from which photomicrographs were made and from which the per cent germination was calculated. The other method involved the transfer of the culture drop with the germinated pollen to a slide. It was then stained with aceto-carmin and mounted in Hoyer's medium, and germination percentage determined by counting those grains forming tubes.

A study of the effect of high temperature and control temperature correlated with the effect of added proline and hydroxyproline on germination percentage was made. Six replications of the treatments were made. Two at the control temperature, with and without 1 mg/10 ml each of proline and hydroxyproline, and two at the high temperature; similarly treated. Table III gives a summary of these results from an analysis of variance evaluated with Duncan's NMRT at the 5% level of significance (Duncan, 1955).

This analysis showed germination to be significantly lower at the high temperature. The addition of proline and hydroxyproline significantly increased germination of pollen from the high temperature treatment over the control and over the high temperature treatment without proline and hydroxyproline. The addition of proline and hydroxyproline, at the concentrations used, significantly decreased germination percentage in the control treatment. There was no significant difference between the high temperature treatment with proline and hydroxyproline added and the control temperature treatment without these amino acids. Likewise, there was no significant difference between the control temperature

with proline and hydroxyproline and the high temperature treatments.

Table III. Summary of analysis of variance of germination percentages.

Rank	Treatment	% Germination
1	Control temperature, 0.1 mg proline and hydroxyproline/ml added	40.0
2	High temperature, proline and hydroxyproline not added	45.7
3	Control temperature, proline and hydroxyproline not added	59.7
4	High temperature, 0.1 mg proline and hydroxyproline/ml added	67.2

Duncan's NMRT at 5% level 13.0, 13.6, 14.0

1 2 3 4

Cytological Observations

Mature pollen from plants grown at the high temperature for fourteen days was smaller in diameter and some contained only a single nucleus (Fig. 1). The microspore mother cells began the initial phases of meiosis. After the nuclear membrane had disappeared, the chromosomes clumped together into many small nuclei. The nucleolus did not disappear. The sporogenous tissue then appeared as multinucleated cells (Fig. 1). In some anthers there were no microspores, but lumps of material which were apparently sporopollenin formed (Fig. 1).

Water Relations

Pollen was collected from twenty-five flowers on one plant at the control temperature. Pollen was collected from the same number of flowers from a plant at the high temperature. The water content at the control temperature was 6.34% and that at high temperature was 4.94%.

When plants were transferred from the greenhouse to a high temperature growth chamber there was a decrease in water content of the inflorescence as seen in Fig. 7. A similar study was made using single buds which were three to four hours from anthesis. After one day there was a remarkable decrease in water content, but from then on there was a steady increase. This was compared with a plant grown continuously under greenhouse conditions (Fig. 8). There was apparently a slightly lower water content of buds which developed first.

In the plants transferred to the high temperature chamber, there was a decrease in dry matter of the inflorescence (Fig. 9). However, the dry weight of single buds from the plant transferred to the high temperature actually increased as seen in Fig. 10.

Pollen was collected from field plots of experimental drought resistant hybrid corn during August of 1966. The pollen was collected at 7:30 a.m. each day and water content determined. As seen in Table IV, the water content of the pollen fluctuated with daily rainfall and temperature.

Fig. 7. The per cent water in the inflorescences of plants grown in the greenhouse and then transferred to a high temperature growth chamber.

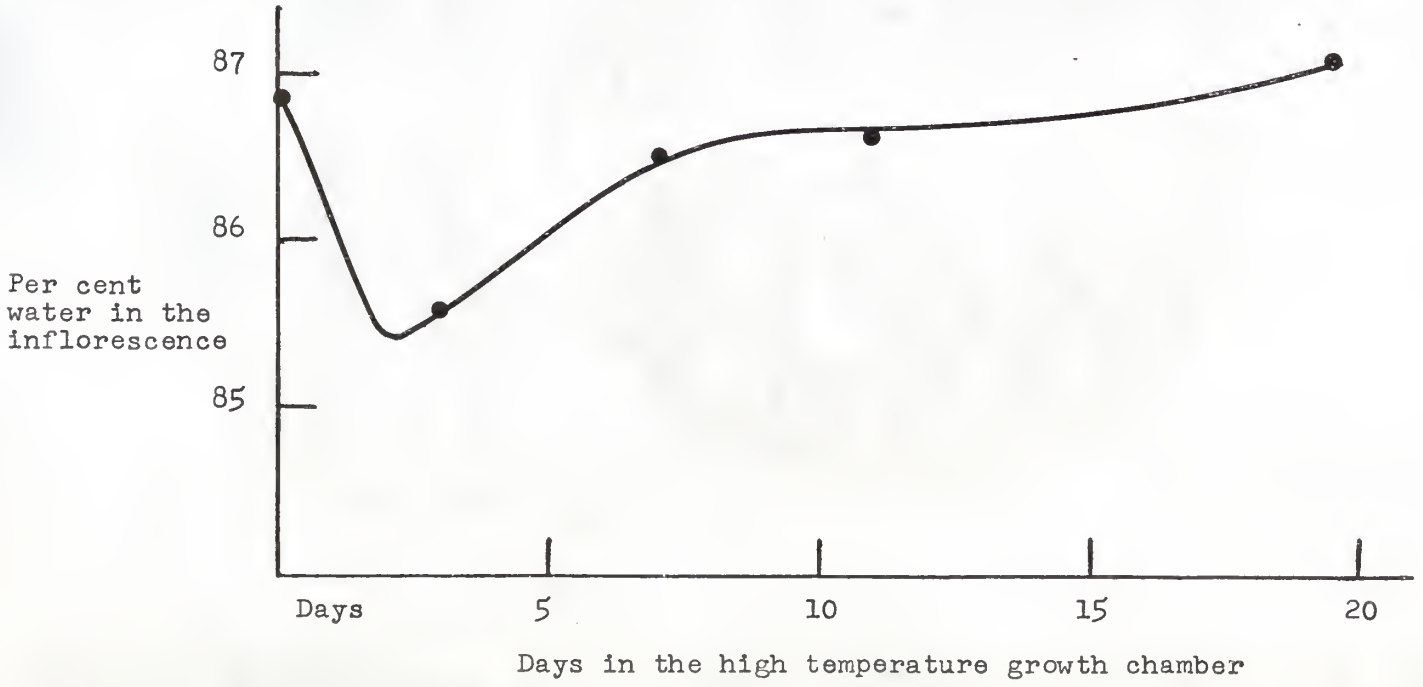


Fig. 8. Comparison of Water Content of Flower Buds Produced by a Plant at Control Temperature with Buds from a Plant at High Temperature.

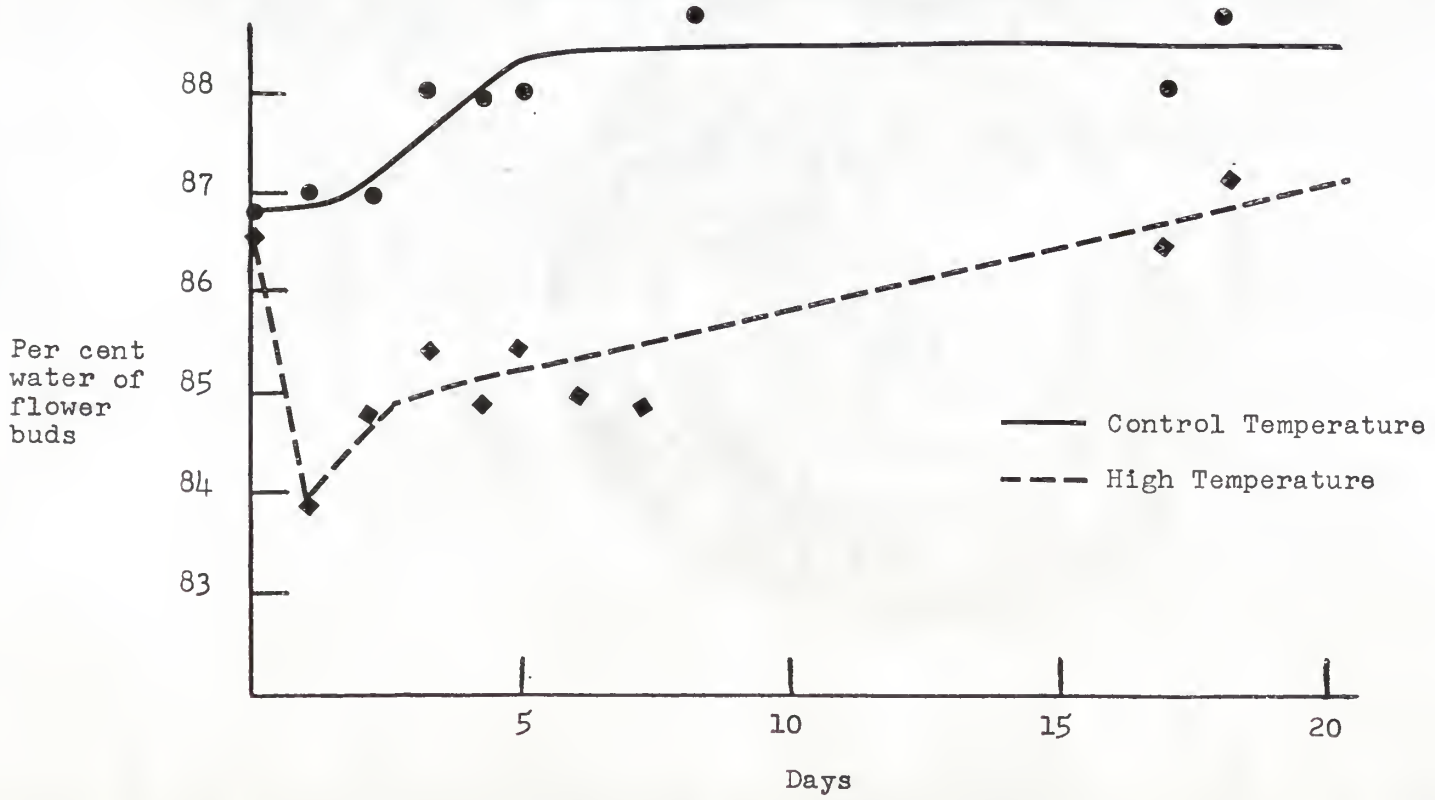


Fig. 9. The decrease in dry weight of inflorescences produced at high temperature.

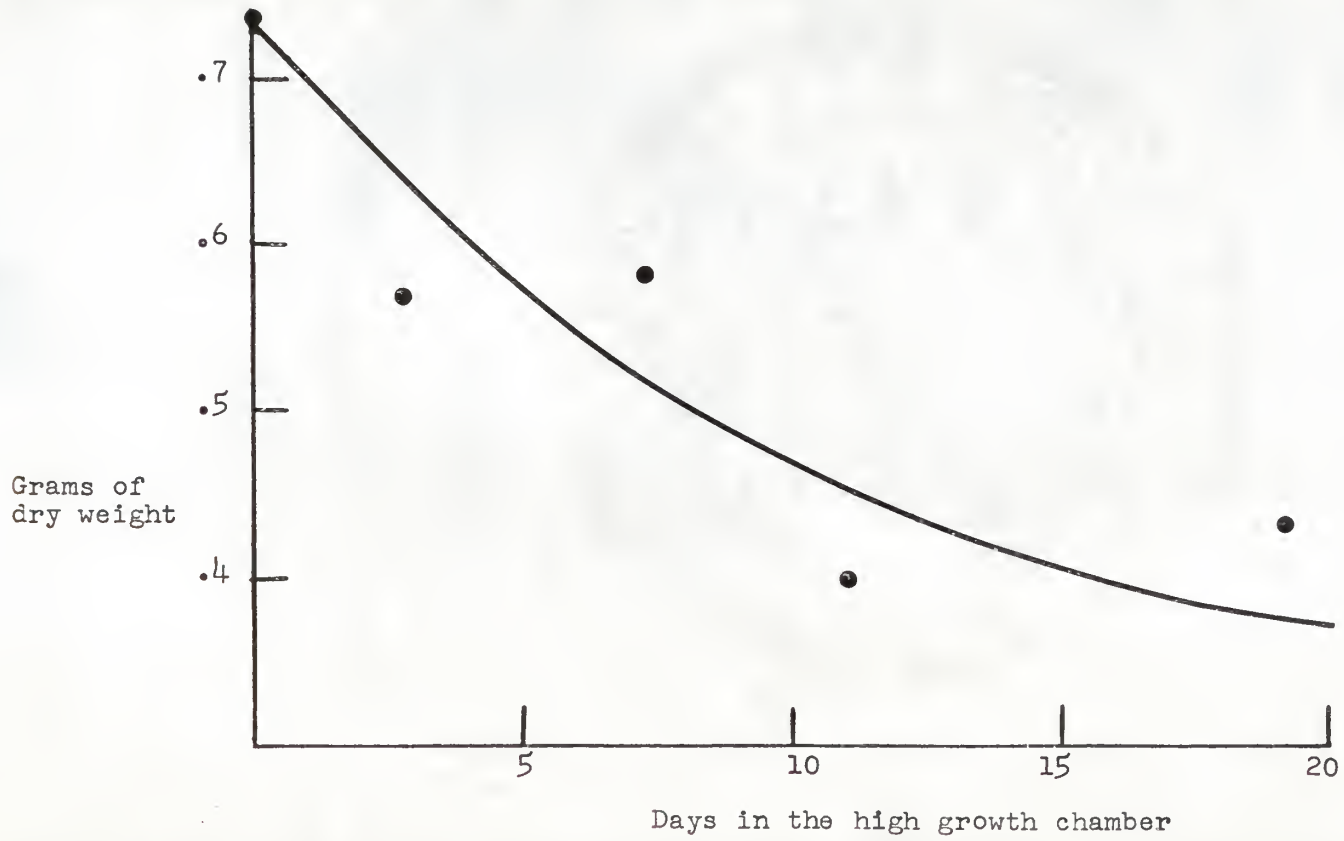


Fig. 10. The dry weights of flower buds at control and high temperatures.

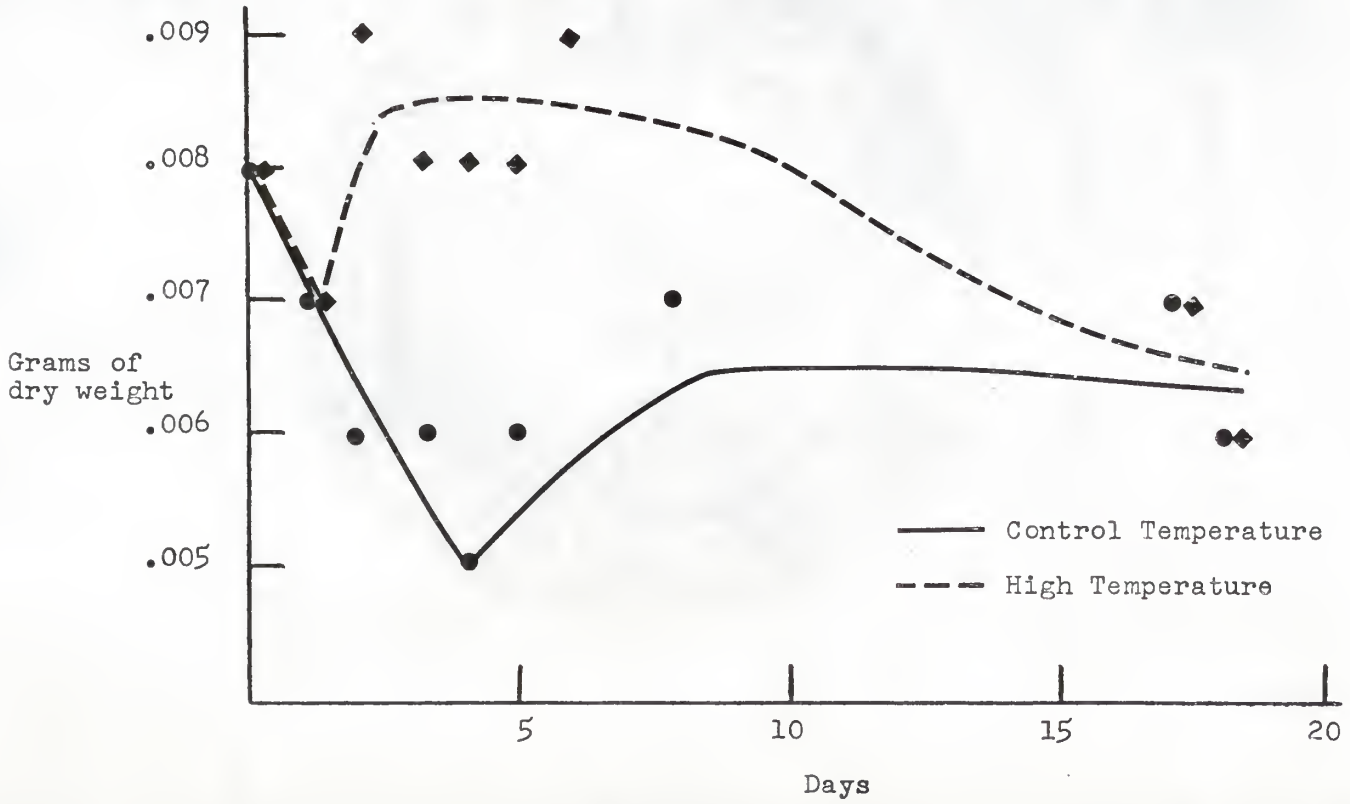


Table IV. Effect of temperature and precipitation on water content of corn pollen.

Date	Temperature Max	Temperature Ave	Precipitation	Per Cent Water
Aug. 1, 1966	94	78	0	-
2	84	74	0.60 in.	28
3	83	72	0	51
4	87	74	0	41
5	90	77	0	46
6	93	79	00	46
7	89	78	0	44
8	78	70	0	44
9	69	65	Trace	54
10	75	68	1.22 in.	49
11	80	67	0	32
12	81	71	0	28
13	79	74	0	-
14	89	74	0	-
15	89	80	0	36
16	95	78	0	-
17	99	84	0	-
18	85	76	0.01 in.	-
19	84	76	0.05 in.	48

DISCUSSION

Pollen development began with the sporogenous tissue differentiating into pollen mother cells which then divide by meiosis to yield a tetrad of microspores, each with one haploid nucleus. Later, these nuclei divide by mitosis to give two nuclei. One nucleus became the tube nucleus and the other became surrounded with cytoplasm and a cell membrane to form the generative cell. Shortly thereafter, anthesis occurred and the pollen grains were released. The duration of these individual stages in pollen grain development varies with species and temperature. In this study, at the control temperature, meiosis occurred about twenty-two days before anthesis and mitosis about eight days before anthesis. However, at the increased temperature associated with the high temperature treatment, the rate of pollen development was accelerated.

The pollen produced after continuous exposure to high temperature was smaller than that produced under controlled temperatures as has also been shown by Schoch-Bodmer (1950), Wagenitz (1955), Kurtz and Liverman (1958), Anikiev (1960), and Kiyosawa (1962).

Pollen viability was reduced at high temperature (Table III). Here the germination percentage was reduced from 59.7% at the control temperature to 45.7% at the high temperature. Similar decreases in pollen viability at high temperatures have been shown with other plant species (See Survey of Literature).

The respiration rate of the pollen grain during germination and tube growth was also altered by high temperatures as indicated

in Tables I and II. Similar studies with pollen have not been reported, but Lvov and Fichtengolz (1936) reported increased rates of respiration by plants suffering from drought injury.

The water content of pollen was altered by high temperature (Table IV), where a reduction in water content is evident. Ornithogalum caudatum pollen from twenty-five flowers at high temperature averaged 4.9% water, and at control temperature contained 6.3% water.

The stage of pollen development at which the high temperature was most injurious appeared to be at the time of meiosis and exine development (Fig. 1, 2, and Table III). Kiyosawa (1962) found that low temperature injury to pollen was also most evident at meiosis and during exine development. During exine development, about the time of microspore mitosis, the microspores have been reported to be most permeable to cytological stains (Stern, 1947). Rowley (1964) also suggested that this is the time substrate is being transferred to the microspores, and Britikov et al. (1964b) believed this to be the time proline was being translocated to the microspores from the vegetative tissues.

Injury to Ornithogalum caudatum pollen at the high temperature treatment used in this study can be alleviated (Table III). In this table it can be seen that adding proline plus hydroxyproline to the germination medium can completely restore the germination percentage that was lowered by the high temperature treatment. Although it is true that at higher temperatures other chemicals may need to be added to overcome heat injury (Langridge, 1963), these results indicate that chemical treatment may alleviate heat injury

to pollen.

SUMMARY

Pollen produced by Ornithogalum caudatum grown in growth chambers programmed for a 31°C fourteen hour day and a 29°C ten hour night was damaged. Initially this temperature created moisture stress in the inflorescence and water content of the pollen was reduced. Associated with this water deficiency, respiratory rate in the pollen was increased. This respiratory activity was apparently associated with hydration of the microspores. The in vitro germination percentage of the pollen was decreased. This decrease in germination can be overcome by addition of 0.1 mg proline and 0.1 mg hydroxyproline/ml of culture medium. Translocation of these amino acids to the pollen or synthesis within apparently failed to occur.

In the earlier developmental stages of flower buds exposed under such conditions, meiosis was apparently inhibited. The microspore mother cells began the initial phases of meiosis, but after the nuclear membrane disappeared, the chromosomes clumped together into many small "nuclei." The nucleolus did not disappear. The cells then appeared multinucleated and further meiotic development apparently did not occur. Diameter of the pollen grains which formed were decreased and single nucleated pollen grains were observed. In some anthers there were no microspores, but lumps of material which were apparently sporopollenin formed.

ACKNOWLEDGEMENT

The author is greatful to the organized government agencies which make education possible. He is personally indebted to his advisor, James A. Goss, Ph.D., Associate Plant Physiologist, for his guidance and stimulation during the course of this study. In addition, he is appreciative of contributions from Mrs. Rosemary Burroughs for assistance in laboratory techniques. He is especially greatful to his wife, Joyce, for her encouragement and cooperation.

LITERATURE CITED

Abaeva, S. S. 1941. Influence of high temperature on viability of pollen in cotton. Compt. Rend. (Doklady) Acad. Sci. URSS 32(6):443-445.

Altergot, V. F. 1960. Fiziologia Ustoichivosti Rastenii. 586: (Izdat. Akad. NaukSSSR).

Anikiev, V. V. 1960. Sbornik Fiziologia Ustoichivosti Rastenii. 441: (Izdat. Akad. NaukSSSR).

Bailey, L. H. 1916. Standard Cyclopedia of Horticulture. IV. New York:The Macmillan Co.

Beatty, J. W., and A. V. Beatty. 1953. Duration of the Stages in Microspore Development in the First Microspore Division of Tradescantia paludosa. Am. J. Botany 40(8):593.

Beeks, R. M. 1955. Improvements in the squash technique for plant chromosomes. El Aliso 3(2):131-133.

Bennet-Clark, T. A. and D. Bexon. 1943. Water relations of plant cells III: The Respiration of plasmolyzed tissues. New Phytol. 42(2):65-92.

Berg, H. V. 1930. Beitrage zur Kenntzis der Pollenphysiologie. Planta 9:105-144.

Bieble, R. 1952. Protoplasma. Vol. 41.

Bolkhavskikh, Z. V. 1965. Nucleic acid changes during development of generative and vegetative cells of the pollen of Lillium. Tsitoloyiya 7:745-748.

Britikov, E. A. and N. A. Musatova. 1964a. Accumulation of free proline in pollen. Fiziol. Rast. 11(3):464-472.

Britikov, E. A., N. A. Musatova, S. V. Vladimirtseva, and M. A. Protsenko. 1964b. Proline in the reproductive system of plants, p. 77-84. In H. F. Linskens, (ed.), Pollen Physiology and Fertilization. Amsterdam:North-Holland Pub. Co.

Duncan, D. B. 1955. Multiple range and multiple F tests. Biomet. 11:1-42.

Erickson, R. O. 1947. Respiration of Developing Anthers. Nature (London) 159(4034):275-276.

Goss, J. A. 1962. In vitro germination of Ornithogalum caudatum pollen. Trans. Kansas Acad. Sci. 65(3):310-317.

Grunvaum, W. B., B. V. Siegel, A. R. Schulz, and P. L. Kirk. 1955. Determination of oxygen uptake by tissue grown in an all glass differential microrespirometer. Mikrochimica Acta. 5-6:1069-1075.

Henckel, P. A. 1946. Tr. Inst. Fiziol. Rast. 5,I.

_____. 1956. Diagnostika zasukhoustoichivosti kulturnykh rastenii i sposoby ee povisheniya (Izadt. Akad. Nauk SSSR, Moskva).

_____. 1964. Physiology of plants under drought. Ann. Rev. Plant Physiol. 15:363-383.

_____, and V. F. Lyubimova. 1947. Uch. Zap. Mosk. Obl. Ped. Inst., 10, Tr. fakult. estestvozn., 3.

Heslop-Harrison, J. 1964. Cell walls, cell membranes and protoplasmic connections during meiosis and pollen development, p. 39-47. In H. F. Linskens, (ed.), Pollen Physiology and Fertilization. Amsterdam:North-Holland Pub. Co.

Hirose, T. 1957. Studies on the pollination of red pepper. I. Flowering and germinability of the pollen. Sci. Rep. Saikyo Univ. 9:5-12.

Johansen, D. A. 1940. Plant Microtechnique. New York:McGraw-Hill Book Co. Inc.

Johri, B. M., and I. K. Vasil. 1961. Physiology of pollen. Botan. Rev. 27:325-381.

Jones, D. F. 1947. Effect of temperature on the growth and sterility of maize. Sci. 105(2728):390-391.

Julander, O. 1945. Drought resistance in range and pasture grasses. Plant Physiol. 20(4):573-599.

Khlevnikova, N. A. 1932. Dokl. Akad. Nauk SSSR, 8.

_____. 1934. Trudy komissii po irrigatsii, 3 (Izdat. Akad. Nauk SSSR).

_____. 1937. Tr. Inst. Fiziol. Rast., Akad, Nauk SSSR, 1, 2.

King, J. R., and T. M. Johnston. 1958. Factors affecting Irish potato pollen germination in an artificial environment. Amer. Potato J. 35:689-700.

Kiyosawa, S. 1962. Influence of some environmental factors on the development of pollen in rice plant. Proc. Crop Sci. Soc Japan 31(1):37-40.

Konarev, V. G. 1959. Nukleinoviya kisloty i morphogenez rastenii (Moskva).

Kruzhilin, A. S., O. A. Zauralov, and A. Y. Mikhalev. 1951. Dokl. Akad. Nauk SSSR, 77:5.

Kurtz, E. B., Jr., and J. L. Liverman. 1958. Some effects of temperature on pollen characters. Bull Torrey Botan. Club 85:136-138.

Langridge, J. 1963. Biochemical aspects of temperature response. Ann. Rev. Plant Physiol. 14:441-462.

Lepeschkin, V. V. 1938. Kolloidchemie des protoplasmas wissen. Forschungsberichte Natur. Reihel 47.

Linskens, H. F. and J. A. M. Schrauwen. 1963. Changes in sulfhydryl content during pollen development. Biol. Plant Acad. Sci. Bohemoscov 5(4):239-248.

Lvov, S. D., and S. S. Fichtengolz. 1936. Exptl. Botan., 2.

Meyer, B. S., D. B. Anderson, and R. H. Bohning. 1960. Introduction to Plant Physiology. New York:D. Van Nostrand Co., Inc.

Nakahara, K., and Y. Komoto. 1957. The affect of high temperature on the meiotic figures on Tradescantia paludosa. Cytologia (Tokyo) 22(1):1-11.

Nasatir, M., A. M. Bryan, and S. D. Rodenberg. 1960. Changes in soluble proteins of developing lily anther. Sci. 897-898.

Ostlind, N. 1945. Investigation concerning pollen germination in artificial substances. Beratt. Alnarps Landtbr.-O Mej Inst. Tradg.:143-172.

Petinov, N. S., and Y. G. Molotkovsky. 1956. Fisiol. Rast., 3(6):516.

_____ and _____. 1957. Fisiol. Rast., 4(3): 225.

_____ and _____. 1960. The effect of respiratory inhibitors on heat resistance in plants. Fisiol. Rast., 7(6):551-556.

Petinov, N. S., and Y. G. Molotkovsky. 1962. Heat stability of plants and ways of increasing it. Vest. Akad. Nauk SSSR 8:62-64.

Roberts, R. H., and B. E. Struckmeyer. 1948. Notes on pollination with special reference to delicious and winesap. Proc. Amer. Soc. Hort. Sci. 51:54-61.

Rowley, J. R. 1964. Formation of the pore in pollen of Poa annua, p. 59-69. In H. F. Linskens, (ed.), Pollen Physiology and Fertilization. Amsterdam:North-Holland Pub. Co.

Satarova, N. A., and G. I. Ulybina. 1960. Sbornik Fisiologia ustoichivosti rastenii, 579(Izdat. Akad. Nauk SSSR, Moskva).

Schoch-Bodmer, H. J. 1940. The influence of nutrition upon pollen grain size in Lythrum Salicaria. J. Genet., 40:393-402.

Sen, B., and G. Varama. 1956. Studies on the pollen of crop plants - mustard and garden - pea. II Proc. 43rd. Indian Sci. Congr. (Agra):247.

Shaw, C. B., and R. S. Loomis. 1965. Ribonucleic acid and protein metabolism in sugar beet drought. Physiol. Plant. 18(1):240-254.

Stern, H. 1947. The permeability of cells undergoing division. Trans. Roy. Soc. Canada, 40(5):141-148.

Tabor, H. 1962. The protective effect of spermine and other polyamines against heat denaturation of DNA. Biochem. 1(3):496-501.

Takats, S. T. 1962. An attempt to detect utilization of DNA breakdown products from the taptum for Lilium longiflorum. Am. J. Botany 49:748-758.

Taylor, J. H. 1959. Autoradiographic studies of nucleic acids and proteins during meiosis in Lilium longiflorum. Am. J. Botany 46:477-484.

Vasil, I. K., and N. Bose. 1959. Cultivation of excised anthers and pollen grains. Indian Botan. Soc., Mem. 2:11-15.

Visser, T. 1955. Germination and storage of pollen. Meded. Landb.-Hoogesch (Wageningen) 55:1-68.

Wagenitz, G. 1955. Changes in the size of cereal pollen under varying nutrition. Ber. deutsch. bot. Ges. 68(8):297-302.

Winkler, A. J. 1926. The influence of pruning on the germinability of pollen and the set of berries in Vitis uninifera. Hilgardia 2:107-124.

SOME EFFECTS OF TEMPERATURE ON THE POLLEN OF *ORNITHOGALUM CAUDATUM*

by

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B. S., Kansas State University, 1965

AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Botany and Plant Patholgoy

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1967

The effects of temperatures above the optimum on pollen development have not been studied in detail. However, physiological and cytological development of pollen has been studied by many workers. In this work, the loss of viability due to high temperatures was studied from cytological observations of pollen development and *in vitro* studies for germination and respiration rates. Respiration rates were determined with a differential ultramicrorespirometer. Liquid hanging drop cultures were employed for germination studies.

Ornithogalum caudatum pollen produced at a 31°C day and a 29°C night temperature was damaged. This temperature created a moisture stress which increased the respiration rate and decreased the germination percentage of mature pollen. This damage occurred during exine development. The decrease in *in vitro* germination was overcome by addition of 0.1 mg proline and 0.1 mg hydroxyproline/ml to the culture medium.

In younger flower buds, meiosis was impaired by high temperature. Diameter of the "pollen grains" was decreased and single nucleated "pollen grains" were observed. In some anthers there were no microspores, but lumps of material which were apparently sporopollenin formed.